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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/867,914	05/30/2001	Neil Andrew Williams	5440US.cip	8062

26850 7590 05/18/2004

MARY M. KRINSKY, Ph. D., J.D.
PATENT ATTORNEY
79 TRUMBULL STREET
NEW HAVEN, CT 06511

EXAMINER	
BORIN, MICHAEL L	

ART UNIT	PAPER NUMBER
1631	

DATE MAILED: 05/18/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/867,914	WILLIAMS ET AL.	
	Examiner	Art Unit	
	Michael Borin	1631	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 March 2004*.

2a)☒ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *50-68* is/are pending in the application.

4a) Of the above claim(s) *54,58 and 59* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *50-53,55-57 and 60-68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Status of Claims

1. Amendment and response filed 03/19/2004 is acknowledged. Claims 10-, 13-49 are canceled. Claims 50-68 are pending. Applicant elected EtxB as agent and insulin as antigen. Claims 54,58,59 are withdrawn from consideration as drawn to non-elected species. Claims 50-53,55-57,60-68 are examined to the extent they read on the elected species.

Priority

2. This application is continuation-in-part of application 09/999458. As the parent application did not address compositions comprising insulin, the claims of instant application drawn to such compositions do not have benefit of earlier filing date of prior application(s) and have priority date of this application, 05/30/2001.

Claim Objections

3. Use of abbreviation GM-1 (claims 50,51,65-68) is noted. For clarity "ganglioside GM-1" is suggested to be used at the first appearance of the abbreviated term.

4. Claims 55,56 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is required to cancel the claims, or amend the claims to place the claims in proper dependent form, or rewrite the claims in independent form.

Claim Rejections - 35 U.S.C. § 112, second paragraph.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 50-53,55-57,60-68 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The rejection is applied for the following reasons:

A. Claim 50,65-68 use term "agent" twice. It is not clear whether there are two different agent, or antibody "agent" is one of the members of Markush group addressed as "an agent". Further, which "said agent" is addressed at the end of the claims? As applicant selected EtxB as agent, the claims are addressed as compositions of EtxB.

B. It is not clear, whether the term "agent" used in the claims means a single compound or a composition.

Claim Rejections - 35 U.S.C. § 102 and 103.

6. Claims 50-53,55-57,60-68 are rejected under 35 U.S.C. 102(b) as anticipated by Uda et al. (US 554378).

The instant claims are drawn to pharmaceutical composition comprising EtxB. Further, dependent claims (claims 53,57) recite antigen, such as insulin, as an additional component.

Uda teaches composition comprising B subunit of Etx and insulin. See claims 1,7,14.

In regard to intended use recited in the claims, arguments related to the intended use of the composition are of little relevance in determining the patentability of the composition. *In re Pearson*, 494 F.2d 1399, 181 USPQ 641 (CCPA 1974). Suggested use limitations do not impart patentability to composition claims where the composition is otherwise anticipated by the prior art.

7. Claims 50,51,52,55,56,60-63 are rejected under 35 U.S.C. 102(b) as anticipated by Ochi et al (CA 2084120; Database Caplus, DN 121:170550), or Amin

et al (Protein Expression and Purification 5, 198-204, 1994), or Wooley et al.(Database Medline, DN 95283386; Annals of the Rheumatic Diseases, 04/1995,54 (4), 298-304)

The instant claims are drawn to pharmaceutical composition comprising EtxB.

The references teach compositions comprising EtxB. See abstracts.

In regard to intended use recited in the claims, arguments related to the intended use of the composition are of little relevance in determining the patentability of the composition. *In re Pearson*, 494 F.2d 1399, 181 USPQ 641 (CCPA 1974). Suggested use limitations do not impart patentability to composition claims where the composition is otherwise anticipated by the prior art. As for the effective dose, the instant claims do not specify the dosage to be used; therefore, the references are assumed to teach the concentration as claimed.

Conclusion.

8. No claims are allowed

9. Applicant's amendment necessitated the new grounds of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Michael Borin whose telephone number is (703) 305-4506. Dr. Borin can normally be reached between the hours of 8:30 A.M. to 5:00 P.M. EST Monday to Friday. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mr. Michael Woodward, can be reached on (703) 308-4028. The fax telephone number for this group is (703) 305-3014.

Any inquiry of a general nature or relating the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.

May 10, 2004

MICHAEL BORIN, PH.D
PRIMARY EXAMINER

PROTEIN EXPRESSION AND PURIFICATION 5, 198–204 (1994)

Purification of the B-Subunit Oligomer of *Escherichia coli* Heat-Labile Enterotoxin by Heterologous Expression and Secretion in a Marine Vibrio

Tehmina Amin and Timothy R. Hirst[1]

The Biological Laboratory, The University, Canterbury, Kent, CT2 7NJ, United Kingdom

Received April 5, 1993

Heat-labile enterotoxins (Etx) are plasmid-encoded, multimeric proteins produced by certain diarrheagenic strains of *Escherichia coli*. The nontoxic, receptor-binding B subunit (EtxB) of such toxins may be useful as a component of vaccines against enterotoxigenic *E. coli*, or as a carrier for the delivery of heterologous epitopes to the mucosal immune system. Here we describe a simple method for the purification of EtxB from a marine vibrio harboring a broad-host range controlled expression vector containing the *etxB* gene. Induction of EtxB resulted in its specific secretion to the medium, to a concentration of greater than 25 mg/liter of culture. The techniques of ultrafiltration and hydrophobic interaction chromatography were used to purify EtxB to homogeneity from the medium of this organism (with a yield of 60.7%). EtxB–epitope fusion proteins were also successfully expressed and secreted in this marine vibrio, suggesting that this system may be of general use in the preparation of EtxB-based vaccines. © 1994 Academic Press, Inc.

Heat-labile enterotoxins (Etx) encompass a group of plasmid-encoded, multimeric protein toxins produced by certain strains of *Escherichia coli* (1). These organisms have been well characterized in their role as pathogens in diarrheal disease, in both man and animals (2–5). Etx are structurally and functionally related to cholera toxin (Ctx) from *Vibrio cholerae* (6,7). Both toxins consist of one A subunit (28 kDa) and five identical, receptor-binding B subunits (11.6 kDa each). The A subunit (EtxA and CtxA) ADP-ribosylates the G protein (GS_α) which regulates the activity of membrane-bound adenylate cyclase. This leads to an accumulation of intracellular cyclic AMP, which triggers increased Cl^- ion secretion and an inhibition of Na^+ ion uptake, resulting in an osmotic imbalance and a concomitant watery diarrhea (8,9). The B subunits (EtxB and CtxB) are nontoxic and mediate binding of the toxin to target cells via the ubiquitous monosialoganglioside receptor, GM1 (10).

CtxB and EtxB are being studied as potential carriers for other smaller molecules (11–14). This may have important implications for the development of combination vaccines and as a means of delivering peptides and other molecules into eukaryotic cells. The recent determination of the three-dimensional structure of Etx (15) will permit advances in the study of the structure–function properties of these toxins (16,17). These developments have highlighted the need for simple methods for the purification of Etx and its component subunits.

Previous methods for purification of Etx or the pentamer of B-subunits ($EtxB_5$) from *E. coli* have been reported (4,18–20). However, because Etx and EtxB normally reside in the periplasm of *E. coli* (21), their purification has required cellular disruption as an essential initial step (18,19). In contrast, in *V. cholerae* Ctx is secreted across both the cytoplasmic and outer membranes to the medium, thus eliminating the need for cell disruption during Ctx purification (22). When genes encoding either Etx or EtxB alone were introduced into *V. cholerae*, these proteins were likewise efficiently expressed and secreted into the medium (23,24). This finding presented the possibility of purifying *E. coli* Etx or EtxB by heterologous production in *V. cholerae* or related organisms. Recently, we demonstrated that a nonpathogenic marine vibrio, *Vibrio* species 60, originally isolated by Oishi and co-workers (25), could serve as a host for the heterologous expression of *E. coli* EtxB (26).

In this paper we report the purification of EtxB from the spent medium of cultures of *Vibrio* species 60 pMMB68. We also show that this organism can be used for the expression and secretion of EtxB fusion proteins; in particular EtxB-$(NANP)_3$ (27), in which the

[1] To whom correspondence should be addressed. Fax: 0227 763912.

1046-5928/94 $6.00

MEDLINE

AN 95283386 MEDLINE

DN 95283386

TI Staphylococcal **enterotoxin** B increases the severity of type II collagen induced arthritis in mice.

AU Wooley P H; Cingel B

CS Department of Internal Medicine, Wayne State University School of Medicine, Detroit, Michigan, USA..

SO ANNALS OF THE RHEUMATIC DISEASES, (1995 Apr) 54 (4) 298-304. Journal code: 62W. ISSN: 0003-4967.

CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals

EM 199509

AB OBJECTIVE--To observe the influence of T cell subset changes on the development of experimental arthritis, by using the bacterial superantigen staphylococcal **enterotoxin** B (SEB) to modulate the T cell repertoire during the arthritogenic response to type II collagen (CII) in vivo. METHODS--DBA/1 mice were injected with SEB before immunisation with CII, and assessed for the development of collagen induced arthritis (CIA) and an immune response to CII. Mice with established arthritis were also treated therapeutically with SEB. Flow cytometry was used to evaluate the effect of the therapy on T cell subsets and T cell receptor (TCR) V beta expression. RESULTS--Mice injected with SEB developed arthritis significantly faster than saline treated control animals, and developed more severe clinical features. Mice treated with SEB after the onset of CIA were also observed to progress more rapidly to a severe arthritis than mice treated with saline alone. The level of anti-CII antibody was not affected by SEB injection. Flow cytometric analysis of TCR expression in mice 21 days after injection of CII showed decreased expression of V beta 6 and V beta 8 cells in SEB treated mice, compared with collagen immunised control mice. Injection of SEB alone caused a decrease in V beta 8, but not V beta 6 T cells compared with the values in normal DBA/1 mice. No significant variations in the T cell repertoire were detected 70 days after CII immunisation. CONCLUSIONS--Treatment with the bacterial **enterotoxin** SEB before the induction of arthritis did not suppress the immunological or arthritogenic response to CII in DBA/1 mice, despite the modulation of the V beta 8 T cell subset. Treatment of mice with established arthritis using SEB provoked a more severe disease course.

L10 ANSWER 4 OF 10 CAPLUS COPYRIGHT 1999 ACS
AN 1994:570550 CAPLUS
DN 121:170550
TI Method using Staphylococcus **enterotoxin** B for treating **autoimmune** diseases
IN Ochi, Atsuo
PA Mount Sinai Hospital Corp., Can.
SO Can. Pat. Appl., 81 pp.
CODEN: CPXXEB
DT Patent
LA English
IC ICM A61K039-085
CC 1-7 (Pharmacology)
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI CA 2084120	AA	19940531	CA 1992-2084120	19921130

AB A method of treating autoimmune diseases (multiple sclerosis, rheumatoid arthritis, etc.) assocd. with a predominance of T-cells expressing V.beta.8+ T-cell receptor comprises administering an amt. of Staphylococcus enterotoxin B (SEB) (or a deriv., analog, or active fragment thereof) effective to reduce the no. and/or inactivate T-cells expressing V.beta.8+ T-cell receptor whereby there is a decrease in disease activity. Methods are also claimed for using SEB to assay for T-cells expressing the V.beta.8+ T-cell receptor assocd. with autoimmune disease pathogenesis and for using SEB to down-regulate lymphokines (preferably tumor necrosis factor and/or interleukin-6).
ST Staphylococcus **enterotoxin** B **autoimmune** disease treatment; T cell **autoimmune** disease **enterotoxin** B
IT Deoxyribonucleic acids
RL: BIOL (Biological study)
(antibody to, redn. of, enterotoxin B of Staphylococcus in, autoimmune disease treatment in relation to)
IT Immune tolerance
(**autoimmune** disease treatment with **enterotoxin** B of Staphylococcus in relation to)
IT Lymphokines and Cytokines
RL: BIOL (Biological study)
(enterotoxin B of Staphylococcus for down-regulation of)
IT Antidiabetics and Hypoglycemics
(enterotoxin B of Staphylococcus, for type I diabetes, V.beta.8+ TCR-expressing T-cell inhibition in relation to)
IT Staphylococcus
(**enterotoxin** B of, **autoimmune** disease treatment with, V.beta.8+ TCR-expressing T-cell inhibition in relation to)
IT **Autoimmune** disease
Lupus erythematosus

Notice of References Cited	Application/Control No. 09/867,914	Applicant(s)/Patent Under Reexamination WILLIAMS ET AL.	
	Examiner Michael Borin	Art Unit 1631	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,554,378	09-1996	Uda et al.	424/434
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N	2084120	05-1994	CA	Ochi	
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Wooley et al. Database Medline, DN 95283386; Annals of the Rheumatic Diseases, 04/1995,54 (4), 298□304
	V	Amin et al Protein Expression and Purification 5, 198_204, 1994
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.